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Original Research Article

Prevalence of Hepatitis E Virus among Hemodialysis Patients: One Egyptian Center Study

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ABSTRACT

Background: In Egypt Hepatitis E virus (HEV) is considered as an endemic infection.
The aim of the present study was to determine the prevalence of HEV infection among HD patients and to study the risk factors associated with such infection.
Methods: The study included ninety six cohort HD patients in Mansoura University Hospital, Egypt in addition to one hundred sixty seven healthy blood donors. Full virological markers assay for hepatitis B, C and E viruses were performed. Positive samples for serological markers for hepatitis E were subjected to nested PCR for HEV.
Results: HCV IgG was the predominant serological markers among HD (42.7%) followed by HEV IgG (22.9%). Serological virological markers for HCV and HEV were significantly higher in HD patients compared to healthy blood donors (18.6%-5.9%, P=0. 0001, P=0. 02 respectively). HEV viremia was detected in statistically significantly higher percent of HD patients (36.4%) compared to blood donors (20%), P=0. 07.
The duration of dialysis and the number of blood transfusion units had no statistically significant association with HEV viremia. HCV antibodies were prevalent among 50% of patients with HEV viremia.
Conclusion: From this study we can reason that HEV is common infection among hemodialysis patients. Further studies are obliged to clear up the wellspring of HEV disease in dialysis units in Egypt.

Keywords: Hemodialysis, HEV, PCR

INTRODUCTION

Hepatitis E virus (HEV) is a non enveloped, positive sense single-stranded RNA virus that is approximately 27 to 34 nm in diameter. It has been classified as the single member of the genus Hepevirus and has a similar structure to the viruses of the *Caliciviridae* and *Tombusviridae* families.

HEV is an etiological agent for enterically transmitted hepatitis mainly in developing countries. [1] It was thought that in developed countries only travelers catch this infection, however, this theory was proven to be non accurate. [2,3] In studies among healthy blood donors in developed countries, seroprevalence of HEV ranges

between 0.4 and 3.2%. [4-6] These findings may suggest the wide distribution of HEV infection.

The transmission of HEV though it is mainly enterically, other modes of transmission have been suggested as parenteral transmission through blood transfusion. [7] There was an association between the presence of hepatitis C pointing to similar or overlapping modes of transmission. [8,9] Like hepatitis A virus, there is a transient viremic stage in the course of HEV infection, which theoretically can be associated with parenteral transmission through blood transfusion. [10]

Patients undergoing regular hemodialysis (HD) are among high risk groups for HEV infections either through blood transfusions or by nosocomial transmission. [11,13]

The aim of the present study was to determine the prevalence of HEV infection among HD patients and to study the risk factors associated with such infection.

MATERIALS AND METHODS

A. Subjects

The study included 96 HD patients recruited from the haemodialysis unit in Mansoura University Hospital, Egypt from June 2013 till August 2014. In addition, one hundred sixty seven healthy blood donors were included in the study.

Each subject participated in the study signed approval consent and the study was approved by the medical ethics committee of Mansoura Faculty of medicine, Egypt.

The most common causes of their renal failure were diabetic nephropathy (n=63), systemic lupus (n=12), Hypertensive nephropathy (n =10), undefined cause *(n* =11).

B. Blood Samples and Serological Markers

Blood samples were drawn from each subject included in the study and serum was separated. For each subject included in the study, serum sample was separated in two aliquots. One aliquot was used for the full biochemical study of liver function tests, including alanine aminotransferase (ALT) aspartate amino transferase (AST) and bilirubin and for routine blood screening tests for hepatitis A, B, C, E and human immunodeficiency virus (HIV). Serological tests included were carried out for hepatitis B surface antigen (HBsAg), hepatitis B core IgM (HbcIgM-BIO-RAD), anti-HCV human immunodeficiency virus (anti-HIV) markers (DIA-Pro, ITALY). Hepatitis A virus IgM and hepatitis E virus both IgM and IgG (HEV IgM-HEV IgG=DSI-EIA-ANTI-HEV-G, ITALY). The second aliquot was stored at -70^{o}C for molecular detection of HEV RNA for subjects with positive IgM or IgG to HEV to detect HEV viremia.

C. Detection of serum HEV RNA by nested RT-PCR

RT-PCR was performed using a QIAGEN One-Step RT-PCR kit according to the manufacturer's instructions. The primers were adopted after *Huang* et al. [14]

Briefly, a reaction tube contained 50 μL of the reaction solutions, including 10 μL of the 5 × QIAGEN One-Step RT-PCR buffer, 2 μL of the dNTP mix (containing 10 mM of each dNTP), 10 μL of the 5 × Q-Solution, 2 μL of the external forward primer (100 pM μL^{-1}), 2 μL of the external forward primer set [5′-AATTATGCC(T)CAGTAC(T)CGG(A)GTTG-3′] and reverse primer set [5′-CCCTTA(G)TCC(T)TGCTGA(C)GCATTCTC-3′] (100 pM μL^{-1}), 2 μL of the QIAGEN One-Step RT-PCR enzyme mix, 1 μL of the RNase Out RNA inhibitor (10 U μL^{-1}; Gibco BRL, Gaithersburg, MD), 10 μL of the template RNA, and 11 μL of the RNase-free water.

The thermal cycling conditions included one step of reverse transcription for 30 min at 50°C and an initial PCR activation

step for 15 min at 95°C. This was followed by 40 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 50°C, and extension for 1 min 15 s at 72°C, and a final incubation for 10 min at 72°C.

A nested PCR was conducted with the following components: 3 μL of the RT-PCR product, 5 μL of the 10 × PCR buffer, 5 μL of $MgCl_2$ (25 mg mL^{-1}), 4 μL of the dNTP mix (10 mM of each dNTP), 1 μL of the nested forward primer [5′-GTT (A) ATGCTT (C) TGCATA (T) CATGGCT-3′]), 1 μL of the nested reverse primer [5′-AGCCGACGAAATCAATTCTGTC-3′] (100 pm μL^{-1}), 0.5 μL of Takara Ex *Taq* polymerase (5 U μL^{-1}), and 30.5 μL of the double-distilled H_2O. The thermal cycling conditions for the nested PCR included 5 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 45°C, and extension for 1 min 15 s at 72°C. This was followed by 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 53°C, and extension for 1 min 15 s at 72°C, and a final incubation for 7 min at 72°C.

Sterile distilled water was used as a negative control. The positive control was the prototype US strain of swine HEV. Positive and negative controls were included in each run with specific molecular weight markers. [14] Strict sterile procedures were followed to avoid false-positive results, such as the use of sterile filter pipette tips, micro centrifuge tubes and the avoidance of carry over of stock solutions.

The amplified PCR products were examined by agarose gel electrophoresis. The expected product of the universal nested RT-PCR was 348 bp.

RESULTS

The study included ninety six patients under regular hemodialysis with mean age 46.6 ± 12.1 years they were 55 males and 41 males.

HCV IgG was the predominant serological markers among HD (42.7%) followed by HEV IgG (22.9%) and HBcIgM (4.2%). While HEV IgM was detected in one patient. Serological virological markers for HCV and HEV were significantly higher in HD patients compared to healthy blood donors (18.6%-5.9%, P=0. 0001, P=0. 02 respectively). (Table 1)

Table (1) Prevalence of hepatitis markers among the studied patients on haemodialysis compared to blood donors

	Dialysis Patients (n=96).	Blood donors (N =167)	P
Sex Male Female	 55(57.3%) 41(42.7%)	 94(59.3% 73 (40.7%)	 P=0. 01
Age Mean± SD	46.6 ± 12.1	30.1± 3.2	P=0. 0001
ALT (mean± SD	30.3 ± 14	30.7± 15	P=0. 61
AST IU/L (mean ± SD)	32.6 ± 17.3	34.2± 16.6	P=0. 97
Bilirubin mg/dl Mean SD	0.95 ± 0.7	0.90 ± 0.6	P=0. 58
Albumin gm/dl Mean± SD	3.5 ± 0.5	3.6 ± 0.5	P=0. 394
HCV IgG	41(42.7%)	31(18.6%)	P=0. 0001
HBcIgM	4(4.2%)	1(0.6%)	P=0. 64
HEV IgG	22(22.9%)	10(5.9%)	P=0. 02
HEV IgM	1(1%)	4(2.4%)	P=0. 0001

HEV viremia was detected in statistically significantly higher percent of HD patients (36.4%) compared to blood donors (20%), P=0. 07, figure 1

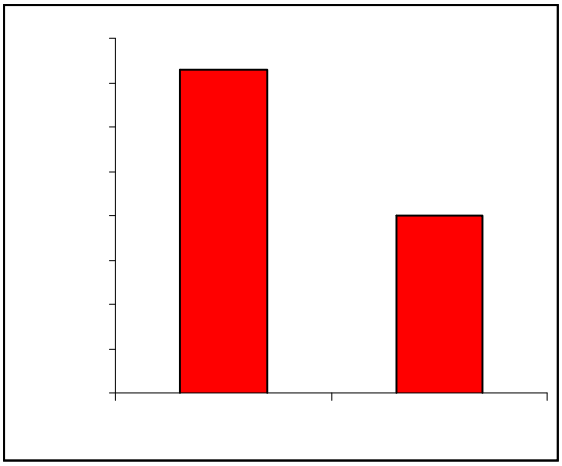

Figure (1): Comparing patients with blood donors for HEV viremia P=0.07

Comparing HD patients with HEV viremia detected by PCR with those negative by PCR, the duration of dialysis, the number of blood transfusion units were higher in patients with HEV viremia than patients negative for HEV viremia, though these values were not statistically significant (P=0. 4, P=0. 3 respectively). HCV antibodies were prevalent among 50% of patients with HEV viremia.

There was statistically insignificant difference between ALT (P=0. 4), AST (P=0. 54) and bilirubin (P=0. 37) between HEV positive HD patients and those negative for HEV viremia. Table 2.

Table (2): Comparative study between HD patients with HEV viremia and those with negative HEV.

	HEV Positive PCR (N=8)	HEV Negative PCR (N=88)	P
Age –Years (mean ±SD)	48.7±12.4	46.1±12.1	P=0. 4
Sex – N0. (%) Male Female	 8 (38.9%) 8 (61.1%)	 47(53.4%) 41(46.6%)	P=0.5
ALT IU/L	27.5± 12.5	30.6± 14.5	P=0.45
AST IU/L	34.5± 20.	32.5± 17.1	P=0.54
Albumin gm/dl	3.8± .35	3.5± .5	P=0.2
Bilirubin mg/dl	.80± 0.1	.96± 0.7	P=0.37
Duration Months (mean± SD)	47.0 ± 35.6	26.7± 23.	P=0.4
Number of Blood units transfusions (mean ±SD)	5.0 ± 1.0	3.8± 2.5	P=0.3
HCV IgG	4(50%)	37(42.04%)	P=0.53

DISCUSSION

Assessment of viral hepatitis among HD patients and looking at those in different focuses in same as well as different nations is a decent list to know the issue of these patients. There are restricted reports about HEV in HD patients in Egypt, however, there are numerous studies about the pervasiveness of HBV and HCV diseases among patients with ESRD in Egypt, yet the discoveries about HEV contamination are constrained. [15]

It gives the idea that the prevalence of HEV disease among patients with HD contrasts from the predominance of HEV in the all inclusive community and it may fluctuate in distinctive hemodialysis units among developed and developing countries. [16-22]

In the present study, the prevalence of HEV IgG was 22.9% among HD patients and 5.9%, among healthy blood donors. Seroprevalence of HEV IgG among HD extended from 1.8 up to 9.8% in studies from distinctive topographical locales. [16,17] Past study from Egypt, reported the prevalence of HEV IgG to be 45.2% (43/95) in blood donors and 39.6% (38/96) in haemodialysis patients. [15] Reports from Egypt about the seroprevalence of HEV in blood donors indicated that prevalence ranges from .45% up to 28.57%. [23,24]

As a rule, the difference of results for the predominance of HEV disease in the overall public at different geological areas could be credited to the criteria for subjects' determination and the courses of HEV transmission. The most paramount variables that assume unmistakable parts in HEV transmission in HD patients are socioeconomic, environmental and intra-unit factors which merit further investigation. [25]

Hepatitis E is a wide spread infection. The transmission of HEV essentially happens by the fecal-oral course, however different courses of transmission legitimately seem to be likely, for example, vertical transmission and blood transfusions, particularly in endemic regions. Patients with end stage renal illness experienced upkeep HD might likewise be at danger of HEV.

HCV antibodies were common among half of patients with HEV viremia in our study. The relationship between HEV and hepatitis C infection (HCV) markers gives off an impression of being hazy, [26,27] past studies had demonstrated a positive serostatus of HBV and HCV in more than 30% of hostile to HEV-positive HD patients. [28] Thus, the relationship between HBV and HCV obtaining and HEV transmission

seems, by all accounts, to be conceivable. Then again, different studies denied the connection between HEV with hepatitis B and C infections. [28-30] It appears that some ecological elements assume a part in keeping up the infection in HD patients like HCV disease, which recommends the vicinity of a typical course of transmission for both infections.

In the present study, there was measurably immaterial distinction between ALT (P=0.4), AST (P=0.54) and bilirubin (P=0.37) between HEV positive HD patients and those negative for HEV viremia

There are civil arguments about the impact of HEV disease on liver chemicals. There were a few studies reporting raised ALT and AST in some HEV-seropositive patients amid intense hepatitis stage, [31,32] our biochemical studies demonstrated no noteworthy contrasts for these two liver chemicals between patients with positive HEV viremic patients and the individuals who are non viremic. Actually, there is quick reduction of the level of these proteins after a time of 1 month post-intense hepatitis top [33] and even sub clinical infections are common with ordinary liver enzymes are regularly connected with HEV diseases particularly in endemic range.

In the present study, there was no relationship in the middle of HEV and the span of HD, number of blood units got and the vicinity of HCV immune response.

There have been reports of HEV infection in patients who got blood transfusions in endemic regions. [29,34-37] Nonetheless, different methods for transmission, for example, individual to-individual and food and water contaminations give off an impression of being a vital course for HEV transmission. Whether sustenance could assume a part as a vehicle in the advancement of hepatitis E episodes in HD focuses still stays hazy. [35-38]

In hate that HEV infection typically have mellow course particularly in Egypt, its relationship with unending hepatitis C has been accounted for to be extreme, [39] watchful observing of such patients alongside hostile to HEV screening at customary interims is proposed. In associated cases with intense hepatitis E, a few studies have prescribed HEV RNA appraisal in serum up to 1 month and in stool up to 6 weeks after the first appearance of the clinical manifestations. [40]

The utilization of molecular methods for the laboratory diagnosis of HEV in HD patients or kidney allograft recipients still stays to be resolved. Be that as it may, our results show the vicinity of HEV viremia in HD patients with positive serological markers in 36.8% and in 20% of blood donors.

This is the first study to our best of information that reports the vicinity of HEV viremia in HD patients. This could signify that molecular procedure for identification of HEV viremia may be connected to serologically positive patients for fitting finding of HEV.

The control of HEV infection in HD units transfer primarily on satisfactory health care workers laborers instruction, application of adequate levels of cleanliness and clean food and water planning. [41] The vitality of preventive cleanliness measures in HEV endemic territories in doctor's facilities particularly in HD units has gotten to be clear since the time of a HEV flare-up in healing center from an intensely contaminated patient.

There is accessible of a HEV recombinant protein vaccine that has been produced to avoid disease. [41] However, its accessibility still remains a significant issue in Egypt. Additionally, the utilization of immunoglobulins for prophylaxis after the exposure to infection have constrained worth. [42,43] Indeed, for the treatment of

HEV disease, no particular antiviral medication has been presented and its treatment has stayed steady so far. [42,44]

From this study we can reason that HEV is common infection among hemodialysis patients. Indeed viremia is a typical finding in patients with positive serological markers for HEV. There is no connection between the length of time of dialysis, blood transfusions or the vicinity of HCV serological markers and the vicinity of HEV viremia. In any case, HEV viremia is not extraordinary among patients with HCV. Further studies are obliged to clear up the wellspring of HEV disease in dialysis units in Egypt.

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